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Toxicity Comparison of Water-Accommodated Fraction and Chemically Enhanced Fraction of Bonny Light Crude Oil and Dispersit SPC 1000 to Mudskipper (*Periophthalmus papilio)* Fish

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ABSTRACT: This study assessed the physicochemical characteristics and toxicity of water-accommodated fraction (WAF) and chemically enhanced WAF (CEWAF) of Bonny Light Crude oil and Dispersit SPC 1000 (dispersant) to Mudskipper fish (*Periophthalmus Papilio*). There were variations in the levels of the physicochemical parameters. The coefficient of variation (CV) indicated that the temperature show the least variation of 2.24% and salinity the highest 98.12%.Hypothesis test for significant differences at $p < 0.05$ significance level however, showed that there were no significant differences in the levels of the measured parameters over the 96 hours. All the mudskippers fish samples exposed to the water accumulated water fraction of 10% bonny oil survived beyond 96 hours. An increase in toxicity of the oil was observed after 72-96 hour exposures of the mudskipper to the oil and dispersant CEWAF. For dispersant concentrations of 540 ml/L and 630 ml/L the survival of mudskippers was 80%. These mortalities might be due to the toxic effect of the dispersant, Dispersit SPC 1000.The Total Petroleum Hydrocarbon (TPH) and the Polycyclic Aromatic Hydrocarbon (PAH) in the test media were respectively 7.66-63.18 ppm and 0.82-5.26 ppm.10% Bonny light crude oil WAF had no effects on mudskipper survival. Exposures to 540 ml/L and 630 ml/L Dispersit SPC 1000 dispersed oil WAF (CEWAF) resulted in 20 % mortality. The lethal dose concentration was 885.5 ml/L (88.55%). The lowest observed effect concentration (LOEC) was found to be 540 ml/L.

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Petroleum hydrocarbons, such as gasoline, are the most abundant organic contaminants in the marine environment (Allen *et al*., 2002). The release of a liquid petroleum hydrocarbon into the environment, especially the marine ecosystem, the ocean or coastal waters, due to human activity, have had disastrous consequences for society, environmentally, economically and socially (Dunnet, *et al.,1982;* Hogan, 2008; Csaba and Csaba 2011; Wout Broekema, 2015; Bautista, and Rahman, 2016). Oil spills have damaging effects on vital habitats such as mangroves, sea grass beds and coral reef areas (Pahila *et al*. 2010). Very low concentrations of dispersants are applied to oil slicks to remove the oil from the sea surface and disperse it into the water column (Lessard and Demarco2000), and to increase biodegradation of oil compounds (Thiem, 1994; Churchill *et al*., 1995; Milinkovich, *et al*., (2011). To reduce the impacts of oil spills to the environment, various measures can be taken such as the application of chemical dispersants. Chemicals dispersants, a common product used to clean and control oil spills in the ocean, are special fluid that bond to the oil molecules and separate them from water molecules, thus breaking up the oil to form tiny oil droplets that can biodegrade more quickly than a mass of oil. The effects of these dispersants and dispersed oil on surface and deep ocean species when dispersing agents are used in spill management have been reported (Tjeerdema, et al.,2000; Qiong, *et al*., 2018; Wong *et al.,* 2006; Guardiola *et al.,* 2012; Onduka *et al*., 2012). Petroleum oil and gasoline may adhere to rocks, plants and animals, or be penetrated into the sediments if a dispersant is used (Allen *et al*., 2002). Increasing coastal oil pollution progressively poses risks to phytoplankton and zooplankton in the marine ecology (Carls *et al*., 2006; Graham *et al*., 2010).

The toxic effects of petroleum hydrocarbon on aquatic organism have been determined (Campbell and Krauss, 2010). These hydrocarbons cause harm to wildlife, and have been found in bivalve molluscs

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taken from the environment (Veerasingam *et al*., 2011; Esler *et al*., 2010). Acute toxicity test of water soluble fraction of diesel fuel on mudskipper (*Periophthalmus koelreuteri)* showed that mudskippers were highly sensitive to very low concentrations of water soluble fraction of diesel fuel (You *et al*, 2018). The toxic effects of bonny oil and Dispersit SPC 1000 dispersant to mudskipper fish (*Periophthalmus Papilio)* have not been extensively studied. These fish, mudskippers, is of economic significance and has a wide distribution; from the west coast of Africa to the whole of Indo-west Pacific Region, and some parts of Persian Gulf (Clayton, 1993; Bu-Olayan and Thomas, 2008; Abdoli, 2010; Neda *et al*., 2013; Amin *et al*., 2003).They have characteristics that make them suitable as a good bio-indicator for biological and eco-toxicological monitoring and assessments of the effects of toxic chemicals in aquatic environment. They are very responsive to ambient environment, and show considerable tolerance to changes in water quality parameters. They are known to absorb and accumulate, in their tissues, higher concentrations of some toxic compounds than other benthic species. (Abid *et al*., 2014; Dabruzzi *et al*., 2011; Polgar *et al*., (2010). The single and joint toxicity of bonny oil and of Dispersit SPC 1000 to this fish have been assessed base on some data from this study (Koranteng-Addo *et al*., 2018) This paper reports the assessment of physicochemical properties and toxicity of water-accommodated fraction (WAF) of Bonny oil, and chemically enhanced WAF (CEWAF) of Bonny oil and dispersant (Dispersit SPC 1000) to mudskippers.

MATERIALS AND METHODS

The Mudskipper fish sample species were obtained from the brackish water research station of African Regional Aquaculture Centre, Buguma, Rivers State, Nigeria. The research was then carried out at laboratory of Pollution Control and Environmental Management Limited (POCEMA) Laboratory at Rumuodomanya, Port Harcourt, Rivers State, Nigeria.

Test Chemicals: Water accommodated hydrocarbon fractions (WAFs) of crude oil were prepared using Bonny Light Crude oil, Yim *et al*., 2011; (2012), which was purchased from NNPC, Port Harcourt, Nigeria. The WAF, CEWAFs were made following a modified Chemical Response to Oil Spills: Ecological Research Forum (CROSERF) methodology Singer *et al*., (2000). In brief the water accommodated fraction (WAF) of crude oil and chemically enhanced water accommodated fraction (CEWAF) of crude oil were prepared under laboratory conditions according to Sikoki and Lelei, (2013), which followed the method of Singer *et al*. (2000) and OECD 2014(4). Briefly, 100 mL of crude oil and 900 mL of water (1:9 oil: water ratio) were added into a 2L side-arm flask. The flask was placed on a magnetic stir plate with a 0.5 in Teflon coated magnetic stir bar. The WAF was spun for a whole day in the dark, with speed such that the vortex formed in the oil was a third of the height of the water. After 24 h the stir plate was turned off and the oil/water mixture was allowed to settle for 12 h for phase separation into an immiscible separating funnel; the bottom layer of the WAF was collected. CEWAF was prepared the same way as WAF, with the exception that after 24 h of stirring, 1:10 ratio of Dispersit SPC 1000 to crude oil, was added (per standard recommendation for dispersant toxicity for maximum effect, Hemmer *et al*., (2010). After 1 h of additional stirring, the stir plate was shut off and the CEWAF was allowed to settle for 12 h, after which time the bottom layer was collected in the same way as the WAF. All treatments were made from the same sample of Bonny Crude oil. Both WAF and CEWAF were prepared fresh for each independent experiment. The mixture was allowed to settle for 12 h for the separation of the water and oil phases. The aqueous layer was drained off and transferred into a clean amber glass bottle and then stored as stock solution. All stocks for the experiment were freshly, because no significant reduction of total petroleum hydrocarbon (TPH) concentration occurred in the WAF stock for 96 h in a preliminary experiment. All concentrations of chemicals tested were made by diluting with filtered mixture of brackish water + distilled water ratio of 1:1. Water qualities such as dissolved oxygen, pH, temperature and salinity in fresh test solutions were recorded before each water change.

Acute toxicity test: Standard static 48 h and 96 h acute toxicity tests using mudskipper fish were conducted following the methods described by Lee *et al*., (2007). The threshold concentration was determined by using the protocols adopted by Hemmer *et al*., (2010), OCED 2014(4), OECD (1992), and OPPTS 850.1075 (1996).

A preliminary range finding test was done on the WAF and CEWAF of the dispersant and crude oil to determine the threshold concentrations at which minimum responses were recorded and concentrations levels noted, (where the anticipation level was expected to be 100% fatality), which was followed by finding a definitive test to determine the LC_{50}.

Then replicates of the test organisms were exposed to different levels of % concentrations of CEWAF, every 24 h during the definitive test water samples and analyzed.

Statistical Analysis: All data analysis was carried out with IBM SPSS version 22 (IBM, Armonk, NY, USA) and XLSTAT module2018, from which the Probit

Analysis was done to determine the lethal concentrations that killed 50% of the population. One way ANOVA was computed to determine the differences in the physicochemical parameters. Statistical significance was assessed at $p<0.05$ level of significance using Kruskal – Wallis Test.

RESULTS AND DISCUSSIONS

The recommended dissolved oxygen levels for protection of freshwater fish range from 3- 8 mg/L for 1-30 days Paul (2010) The generally accepted minimum amount of dissolved oxygen that will support a large population of various fishes is from 4 to 5 mg/l. Below 3 mg/L even the resilient fish die Oram (2014). In this study the dissolved oxygen content of the media ranged from 2.59 - 6.5 mg/l. Some exposure media had levels below 3mg/L. This means some fishes receiving low oxygen 2.59 mg/L may be stressed or die beyond 24 hours of exposure**.** The descriptive statistics of the physicochemical parameters for the study tanks measured daily for four days (96 hours) are shown in Table 2. There were variations in the levels of the physicochemical parameters. The coefficient of variation (CV) indicated that the temperature show the least variation of 2.24% and salinity the highest 98.12% (Table 3).

Table 1: The mean of replicate measured for physicochemical parameters for the various water media

S/ No	PARAMETERS	Saline Water and Fresh Water	Brackish Water	Fresh Water	WAF	CEWAF
1	pH	6.83± 011	7.44±00	4.43±01	7.01±01	6.0±09
2	Temperature °C	26.7±0.01	27.3±0.01	26.8±0.01	26.2±0.01	29.1±0.00
3	Electrical Conductivity, EC (µS/cm)	17.40±05	41.6± 2	0.23± 0.0	0.05±0.00	0.07±0.00
4	Total Dissolved Solid, TDS (ppm)	8.62±02	25.00.±01	110. ±0.93	40±0.05	40.0±0.03
5	Specific Gravity, S.G	1.008± 0.01	1.02±0.001	1.00±0.00	-	-
6	Salinity (mg/L)	17,655.00	21,450 ±1.1	146.0± 1.2	37.95± 0.9	183.15±
7	Alkalinity (mg/L)	40.0±0.09	340± 0.01	40.00±0.9	20±0.01	20±0.00
8	Dissolved Oxygen, DO (ppm)	5.70±0.02	2.59±0.09	5.11±0.04	4.75±0.10	6.50±0.01
9	BOD (ppm)	4.17±0.03	0.14±0.01	0.28±0.04	1.41±0.00	5.41±0.00
10	Hardness (mg/L)	2,000±0.7	6,000 ±01	40.00±01	40±0.10	20±0.01

Table 2: Descriptive statics of physicochemical parameters of media used for toxicity test

	Concentration / ml /L					
Parameter	0	270	360	450	540	630
pH	6.90±0.49	6.73±0.30	6.51±0.40	6.41±0.44	6.52±0.40	6.41±0.41
Temp. ^{0}C	27.84±0.12	28,26±0.13	28.08±0.13	28.10±0.13	28.40±0.13	28.36±0.13
Salinity /µS/cm	10445.82±52	10044±53	10576±50	9317±56	11964±56	9854±37
EC/ppm	10.16±2.55	9.08±0.73	8.24±0.44	6.80±0.97	6.39±0.85	5.65±0.39
TDS/ mg/L	5056±1271	3518±953	3290±856	2753±851	2560±650	2290±599
Alkalinity/ mg/L	60±6.32	52.8±8.0	53±8.0	56,7±7.48	52.8±8.0	56.4±12
Hardness/mg/L	2440±371	1560±147	1520±80	1200±153	960±98	889±80
DO / ppm	4.21±0.39	4.94±0.32	3.91±0.68	3.79±0.77	3.16±0.86	2.88±0.91

Table 3: Variations in Physicochemical Parameters

Parameter	N	Range	Min.	Max.	Mean	Std. Error	Std. Deviation	(CV) %
pH	30	2.75	5.00	7.75	6.58	0.16	0.85	12.91
Temperature /^{0}C	30	3.30	26.40	29.70	28.17	0.12	0.63	2.24
Salinity / mg/L	25	28672.05	367.95	29040.00	10370.18	2035.17	10175.84	98.12
Alkalinity / mg/L	30	60.00	40.00	100	54.67	3.17	17.37	31.09
DO / ppm	30	5.13	1.25	6.38	3.82	0.29	1.57	41.10

Hypothesis test for significant differences at $p < 0.05$ significance level however, showed that there were no significant differences in the levels of the measured parameters over the 96 hours (table 4) .

Thus no difference was observed between exposure conditions. The mud skippers survived in these water media over 96 hour period in-spite of the varied levels of these physicochemical properties. All the mudskippers fish samples exposed to the water accumulated water fraction of 10% bonny oil survived beyond 96 hours, even though the dissolved oxygen content of some exposure tank for the WAF were below 3 mg/L. The fish, however, has been observed to be highly sensitive to very low concentrations of water soluble fraction of diesel fuel Yim *et al*.,(2012). The no observable adverse effect or mortality that was observed in this study probably, may be as a result of due to accumulation by the mudskippers of some toxic hydrocarbons from oil, PAHs, which did not exceed their body burden, a critical internal threshold level, the concentration at which 50% of the individuals would be negatively affected McCarty, 2010), as they are adapted to the polluted oil mangrove environment, the Niger Delta estuary, from where they were sampled. Preliminary analysis of Total Petroleum Hydrocarbon (TPH) in the water samples was between 3.96- 36.22 ppm. (Data not shown). Mudskippers have been observed to accumulate very high concentrations of toxic compounds in their tissues Polgar *et al*., (2010), and breathe comfortably, in water as well as on land, in air You, 2014; Graham,(1997), this could also account for their survival in the WAF of bonny oil, and also under limited dissolved oxygen in the WAF. The no effects observed for survival of mudskippers is not strange. Van Scoy *et al*., (2012), Milinkovitch *et al*., (2012) observed survival of both fish and embryos was not affected by 96 h spike exposures to water-accommodated fractions (WAFs) of physically- dispersed weathered Prudhoe Bay crude oil (PBCO). The toxicity 10% of bonny oil used was probably low. However, the Bonny Light crude oil is toxic to Sarotherodon *melanotheron* (Seiyaboh, *et al*., 2013; Ayoola and Alajabo (2012). Table 5 shows the toxic effect of the chemical enhanced water accumulated water fraction (CEWAF) of bonny oil to mud skipper. An increase in toxicity of the oil was observed after 72-96 hour exposures of the mudskipper to the oil and dispersant CEWAF. For dispersant concentrations of 540 ml/L and 630 ml/L the survival of mudskippers was 80%. These mortalities might be due to the toxic effect of the dispersant, Dispersit SPC 1000. The observation of this study agrees with other studies Ozhan *et al*., 2014; Cohen *et al*., 2014; Anderson *et al*., 2014; Baek *et al*., 2013; Lee *et al*., (2013). Whereas the survival of mudskipper fish was not affected by 96 hour exposures or CEWAF Van der Geer, (2010), and also did not cause synergistic toxicity to fish embryos (Adams *et al*., (2013), this study noticed effect on mudskipper survival.

Table 4: Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of pH is the same across categories of concentration	Independent samples. Kruskal-Wallis Test	0.707	Retain the null hypothesis
2	The distribution of temperature is the same across categories of concentration	Independent samples. Kruskal-Wallis Test	0.724	Retain the null hypothesis
3	The distribution of salinity is the same across categories of concentration	Independent samples. Kruskal-Wallis Test	0.997	Retain the null hypothesis
4	The distribution of alkalinity is the same across categories of concentration	Independent samples. Kruskal-Wallis Test	0.918	Retain the null hypothesis
5	The distribution of dissolved oxygen is the same across categories of concentration	Independent samples. Kruskal-Wallis Test	0.300	Retain the null hypothesis

****The Asymptotic significance are displayed. The significance level is 0.05.*

Table 5: Acute Toxicity of Chemical Enhanced Water Accumulated Water fraction (CEWAF) to Mud skipper

Exposure time (Hours)	Percent mortality of mudskipper exposed to various concentration of Dispersit SPC 1000 CEWAF				
	270 (ml/l)	360 (ml/l)	450 (ml/l)	540 (ml/l)	630 (ml/l)
24	0	0	0	0	0
48	0	0	0	0	0
72	0	0	0	20	20
96	0	0	0	20	20

Total Petroleum Hydrocarbon (TPH) and the Polycyclic Aromatic Hydrocarbon (PAH) in the test media were respectively 7.66-63.18 ppm and 0.82-5.26 ppm. Even though the addition of the dispersant increased the concentrations of the toxic PAHs and TPH as well as the toxicity of the oil (Ozhan *et al*.. 2014; Baek *et al*., (2013), the toxic effect observed could be attributed to the dispersant as exposure of the fish to 5% v/v (50 ml/L) of the dispersant only in the water media used for the study caused 100% mortality in 96 hours and no mortality observed for exposure to bonny oil only Lee *et al*., (2013). The lowest concentration of the dispersant in 10% WAF of bonny oil that resulted in observed effect (LOEC) was 540 ml/L. The probit mortality analysis of the CEWAF for 24-96 hour exposure indicated that the lethal dose concentration LC_{50} was 88.55 % ($y = -4.635x+14.02$). Thus 885.5 ml/L of dispersant in 10% bonny oil WAF would result in 50% mortality.

Conclusion: In summary, the results indicated the low toxicity of Bonny Light Crude oil (WAF) and relatively higher toxicity of Dispersit SPC 1000 dispersed oil WAF (CEWAF) to mudskipper fish. Information generated from this research can be used in understanding the effect of Dispersit SPC 1000 to mudskipper.

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